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Dasatinib targets c-Src kinase in cardiotoxicity

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ABSTRACT

Dasatinib is a multitargeted kinase inhibitor used for treatment of chronic myeloid leukemia and acute lymphoblastic leukemia. Unfortunately, treatment of cancer patients with some kinase inhibitors has been associated with cardiotoxicity. Cancer treatment with dasatinib has been reported to be associated with cardiotoxic side effects such as left ventricular dysfunction, heart failure, pericardial effusion and pulmonary hypertension. Here we aimed to investigate the molecular mechanisms underlying the cardiotoxicity of dasatinib. We found that among the resident cardiac cell types, cardiomyocytes were most sensitive to dasatinib-induced cell death. Exposure of cardiomyocytes to dasatinib attenuated the activity of extracellular signal-regulated kinase (ERK), which is a downstream target of dasatinib target kinase c-Src. Similar to dasatinib, c-Src depletion in cardiomyocytes compromised cardiomyocyte viability. Overexpression of dasatinib-resistant mutant of c-Src rescued the toxicity of dasatinib on cardiomyocytes, whereas forced expression of wild type c-Src did not have protective effect. Collectively, our results show that c-Src is a key target of dasatinib mediating the toxicity of dasatinib to cardiomyocytes. These findings may influence future drug design and suggest closer monitoring of patients treated with agents targeting c-Src for possible adverse cardiac effects.

1. Introduction

Small molecule tyrosine kinase inhibitors have become a mainstay in the treatment of various cancers. There are currently over 2300 clinical trials ongoing with kinase inhibitors (KIs), and while the vast majority of those are investigating the use of kinase inhibitors for treatment of malignancies, they are also studied for the treatment of numerous other diseases, including respiratory tract diseases, immune system diseases and nervous system diseases. KIs typically target multiple kinases and, in addition to tyrosine kinases, a number of these agents target various serine/threonine kinases in mammalian cells. Unfortunately, treatment of cancer patients with some of the KIs has been associated with cardiotoxicity [1–5]. The main cardiovascular side effects include hypertension, myocardial ischemia, left ventricular (LV) dysfunction and heart failure (HF). Routine monitoring for adverse cardiac effects of KIs currently mainly relies on assessment of LV function by echocardiography [6]. Analysis for circulating biomarkers, such as troponin I, troponin T and NT-proBNP may also have prognostic value [7,8]. However, it remains a clinical challenge to detect or predict cardiotoxicity prior to manifestation of adverse cardiac effects. This raises a need to better understand the mechanisms of KI toxicity that would enable the design of KIs with optimal target kinase profile in order to avoid cardiotoxic effects. Cardiac injury may lead to congestive heart failure (HF), and patients with HF have fatigue, marked limitation of physical activity and dyspnea, and one third of patients with severe heart failure require hospitalization within three months of diagnosis. Despite therapies available, clinically manifest severe heart failure has poor prognosis with 5-year mortality rate of 50% [9].

There are currently 68 KIs approved by the FDA [10]. The first KI approved was imatinib, which targets Bcr-Abl, an oncogene caused by chromosomal translocation (known as the Philadelphia chromosome, Ph) and a causal factor in 95% of cases of chronic myelogenous leukemia (CML) [11]. In addition, imatinib inhibits a number of other kinases, including the receptor tyrosine kinases platelet-derived growth factor receptor α and β (PDGFR α/β), mast/stem cell growth factor receptor Kit, epithelial discoidin domain-containing receptors 1 and 2 (DDR1/2),

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colony stimulating factor 1 receptor (CSF1R) and leukocyte C-terminal Src kinase (LCK) [12]. The non-receptor tyrosine kinase c-Src was the first protein kinase identified to phosphorylate tyrosine residues [13]. Hence, 10 other Src family of kinases have been identified. c-Src (Src from here on) is activated by various growth factors and cytokines and regulates multiple cellular functions, including cell differentiation, proliferation and survival [14]. Depending on the interacting partner, Src may activate multiple downstream targets, including PI3K-Akt, Ras-Raf-MEK-ERK1/2-MAPK, STAT3 or p190RhoGAP to exert biological effects [15–18].

Dasatinib (BMS-354825) is a second-generation KI that is used for the treatment of patients with Philadelphia-chromosome-positive (Ph+) chronic myelogenous leukemia and Ph+ acute lymphoblastic leukemia. It is a potent inhibitor of Bcr-Abl and Src kinases, and additionally inhibits e.g., PDGFR α/β, LCK, ephrin type-A and type-B receptor kinases and tyrosine-protein kinase Blk [12]. Fluid retention is a common side effect of dasatinib and the reported adverse cardiovascular effects for dasatinib include LV dysfunction, heart failure, pericardial effusion, pulmonary hypertension, QT interval prolongation and arrhythmias [19–21]. Interestingly, previous studies have identified a gatekeeper mutation T338I in Src gene (T341I in human), which blocks dasatinib binding to its kinase domain and renders Src resistant to dasatinib [22–24].

While the use of dasatinib is associated with cardiovascular side-effects, the mechanisms of the toxicity are poorly understood. Here, the toxicity of dasatinib was analyzed in major resident cardiac cell types and the underlying mechanism of toxicity was explored in cardiomyocytes. We find that dasatinib reduces cardiomyocyte viability that is associated with reduced phosphorylation of ERK. Our experimental data identifies Src, an upstream regulator of ERK, as a key downstream target of dasatinib regulating cardiomyocyte viability.

2. Methods

2.1. Cell cultures

Neonatal rat ventricular cardiomyocytes were isolated by a collagenase dissociation method as described earlier [25]. Experimental protocol was approved (35/2019, February 10th, 2020) by the Animal Use and Care Committee in the University of Oulu and experimentation conducted in accordance with the national regulations on the usage and welfare of laboratory animals. Briefly, 2–4 days old Sprague Dawley rat pups were decapitated, the thoracic cavity was opened, and the isolated ventricles were digested in 2 g/l collagenase solution in PBS, supplemented with 50 μM $CaCl_2$ at 37 °C. Cells were resuspended in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS, Gibco # 10500–064) and antibiotics (100 IU/ml penicillin and 100 mg/l streptomycin). The suspension was pre-plated in 100 mm dishes and incubated for 2 h at 37 °C in humidified atmosphere with 5% CO_2 to remove the nonmyocyte cell fraction. The non-attaching cardiomyocytes were collected and plated on 96-well plates at a density of 60 000 cells/well. The medium was changed the following day to Ham's F10 Nutrient Medium (Invitrogen #31550–023) supplemented with 2% FBS and antibiotics for another 24 h before the experiment started.

Primary human umbilical vein endothelial cells (HUVECs), obtained from Cell Applications, were maintained in culture up to passage 8. For experiments, cells were plated at 10,000 cells/well in 96-well cell culture plates in endothelial cell growth medium purchased from Cell Applications. The medium was changed 24 h after plating and every 48 h until the cells reached sufficient confluency.

Human cardiac fibroblasts were obtained from ScienCell (#6300) and cultivated in Fibroblast Medium-2 (ScienCell #2331) supplemented with 5% fetal bovine serum (ScienCell #0025), 1% fibroblast growth supplement-2 (ScienCell #2382) and penicillin/streptomycin solution (ScienCell #0503). For experiments, fibroblasts were plated 2500 cells/well in 96-well plates and experiments were started the following day.

Dasatinib (LC Laboratories) was dissolved into DMSO as a stock concentration of 500 μM. This was further diluted into cell culture medium to achieve the final concentration. The final DMSO concentration was set constant in all experimental groups (≤0.2%). Fibroblast growth factor (Peprotech) was resuspended into an aqueous buffer according to manufacturer's instructions and applied to cells at a concentration of 20 ng/ml.

2.2. Cell viability and cell death assays

For the analysis of cell viability, ATP assay (Promega #G7570) was performed to determine the percentage of viable cells in the culture after treatment with different concentrations of dasatinib. To validate the assay, we plated cardiomyocytes onto 96-well dish with normal density and then stepwise reduced the number of cells plated down to 30% of the normal density. We then immediately analyzed for ATP levels in these cells and found that it highly correlated with the number of cells plated ($r2 = 0.99$). Thus, ATP assay provides a linear analysis for measuring the proportion of viable cardiomyocytes.

For the analysis of necrotic cell death, the Toxilight assay (Lonza #LT07–117) was used to detect the release of adenylate kinase from ruptured cells.[4] Adenylate kinase is an ubiquitous enzyme present in different cell types and plays a key role in cellular energy balance and conversion of adenosine phosphates. Adenylate kinase is released from dying cells, allowing for repeated collection of medium samples from the cell culture. Samples were scanned for luminescence on 96-well plate with a multiplate reader (Varioskan ThermoFisher).

2.3. Western blot

Cells were lysed in 20 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton-X100, 2.5 mM sodium pyrophosphate, supplemented with 1 mM β-glycerophosphate, 1 mM Na_3VO_4 (pH 7.5), 1 mM dithiothreitol, protease inhibitors (Sigma #P8340), and phosphatase inhibitors (Sigma #P0044). Protein extracts were matched for protein concentration, loaded on SDS-PAGE, and transferred to nitrocellulose membranes. The membranes were blocked in Odyssey blocking buffer (LI-COR Biosciences), incubated with primary antibodies overnight at 4 °C. The membranes were incubated with secondary antibodies (1:5000, LI-COR Biosciences) for 1 h at RT and protein levels were detected using an Odyssey Fc imaging system (LI-COR Biosciences). Blots were quantified with Image Studio software (LI-COR). Primary antibodies against phospho-Akt (Ser473) (Cell Signaling #9271, 1:1000), phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling #9106, 1:2000), Src (32G6) (Cell Signaling #2123, 1:1000) p-STAT3 (Cell Signaling #9134, 1:1000), and GAPDH (Millipore #MAB374, 1:500 000) were used.

2.4. RNA interference and plasmids

Specific Src siRNA (ThermoFisher ID s136282) and negative control siRNA (ThermoFisher/Dharmacon siGENOME Non-Targeting siRNA Control Pool D-001206–13–50) were transfected into cardiomyocytes using Lipofectamine2000 as a transfection reagent. The cells were incubated with siRNAs in OPTImem medium for 18 h. Thereafter the cardiomyocytes were incubated in Ham's F10 Nutrient Mix medium supplemented with 2% FBS. The cells were collected for biochemical analyses 4–6 d after transfection.

Wild type c-Src (pBABE-Src-Rescue, Addgene plasmid # 26983; http://n2t.net/addgene:26983; RRID:Addgene_26983) and dasatinib resistant c-Src (pBABE-Src-Dasatinib-resistant, Addgene plasmid # 26980; http://n2t.net/addgene:26980; RRID:Addgene_26980) were a gift from Joan Massague.[26] The plasmids were transfected into cardiomyocytes using Lipofectamine2000 and the cells were collected for biochemical analyses 3–5 d after transfection.

2.5. Statistical analysis

IBM SPSS Statistics software (IBM, Armonk, NY) was used for determining statistical significance. Normally distributed data was analyzed with one-way analysis of variance followed by Dunnett's or Tukey's post hoc test. When two groups were compared, Student's t-test was used. Two-way repeated-measures ANOVA was used to evaluate the statistical significance of differences in cardiomyocyte death following c-Src silencing.

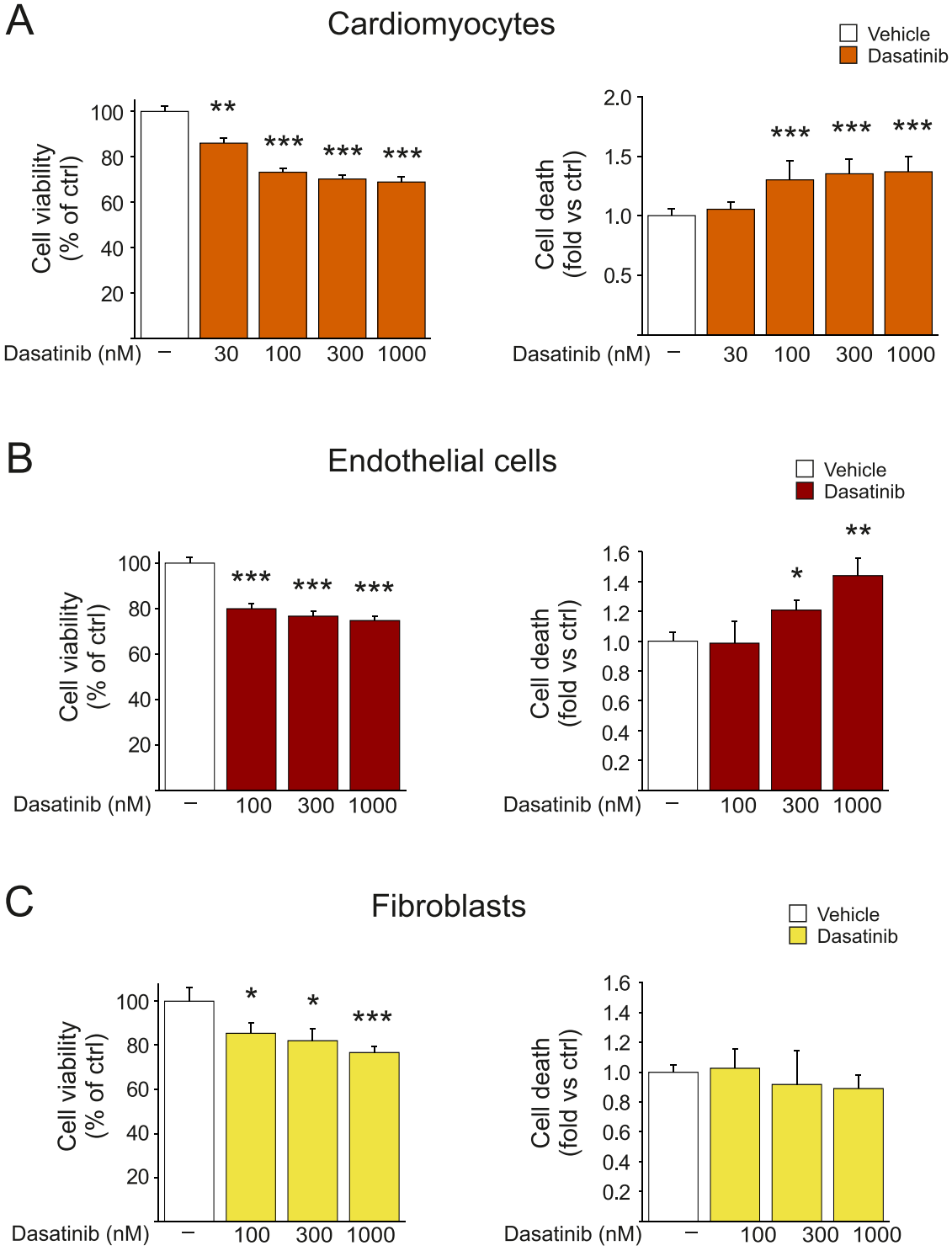

Fig. 1. *Toxicity of dasatinib on resident cardiac cells* A. Neonatal rat ventricular cardiomyocytes were treated with different concentrations of dasatinib for 24 h. Shown are analysis for cardiomyocyte viability by ATP assay (left panel) and analysis for cell death by measuring the release of adenylate kinase from ruptured cardiomyocytes (right panel). N = 6. B. Human umbilical vein endothelial cells treated with dasatinib for 24 h. Shown is analysis for cell viability by ATP assay and analysis for cell death by measuring the release of adenylate kinase. N = 6. C. Human cardiac fibroblasts treated with dasatinib for 24 h. Shown is analysis for cell viability by ATP assay and analysis for cell death by measuring the release of adenylate kinase. N = 6. *p < 0.05, * *p < 0.01, * **p < 0.001 vs Vehicle. Statistics were calculated by one-way analysis of variance followed by Dunnett's post hoc test.

3. Results

3.1. Dasatinib induces a dose-dependent decrease in cardiomyocyte viability

Neonatal rat cardiomyocytes were treated with 30–1000 nM concentrations of dasatinib for 24 h and the cell viability was assessed by measuring cellular ATP levels and cell membrane integrity by adenylate kinase assay. ATP assay showed a dose dependent decrease in cardiomyocyte viability after dasatinib exposure (Fig. 1A). Interestingly, the highest toxicity was observed with 300 nM dasatinib, while 1000 nM dasatinib caused only modest further decrease in cellular ATP levels. Additionally, we analyzed for cardiomyocyte death by measuring the release of adenylate kinase to cell culture medium from ruptured cardiomyocytes. Here, dasatinib induced a dose-dependent increase in cardiomyocyte death (Fig. 1A) and, similar to the ATP assay, maximal cell death was achieved with 300 nM dasatinib.

3.2. Analysis for the effect of dasatinib on endothelial cell and fibroblast viability

To investigate if dasatinib induces toxicity in other major cardiac cell types, we analyzed for the toxicity of dasatinib on endothelial cells and fibroblasts. Treatment of HUVECs with dasatinib induced a dose-dependent decrease in cellular ATP levels (Fig. 1B). The decrease in ATP levels was detected at 100 nM dasatinib and was not substantially augmented by increasing the concentration from 300 nM to 1000 nM. Adenylate kinase assay indicated an increase in cell death with 300 nM and 1000 nM dasatinib, whereas exposure of endothelial cells to 100 nM dasatinib did not induce cell death (Fig. 1B).

Treatment of human cardiac fibroblasts with dasatinib induced a dose-dependent decrease in cellular ATP levels (Fig. 1C). However, the release of adenylate kinase was not detected even with 1000 nM dasatinib (Fig. 1C).

The studies in different cardiac cell types thus indicate that treatment of cardiomyocytes with dasatinib reduces ATP levels that also culminates in cell death. Similarly, the decrease in endothelial cell ATP levels reduces cell viability, but the cardiomyocytes are more sensitive to dasatinib-induced cell death compared to endothelial cells. In fibroblasts, the decrease in ATP levels does not appear to induce necrotic cell death.

3.3. Analysis for the potential target of dasatinib mediating the toxicity to cardiomyocytes

There is prior data showing that dasatinib induces toxicity to endothelial cells and the mechanisms of the toxicity are at least partially known [27]. On the other hand, the mechanisms of cardiomyocyte toxicity of dasatinib have not been explored. Western blot analysis of cardiomyocyte samples showed that dasatinib had no effect on basal or FGF-induced STAT3 or Akt phosphorylation (Fig. 2A). On the other hand, dasatinib showed potential in decreasing both basal and FGF-induced ERK phosphorylation (Fig. 2A).

Dose-response curve derived from cell viability assay data in cardiomyocytes shows that dasatinib induces a 20% decrease in cardiomyocyte viability with 40 nM concentration (Fig. 2B). Kinase profiling data with dasatinib reveals 84 kinases targeted by dasatinib in the primary screen [28]. However, there are 42 kinases which have a binding constant below 40 nM with dasatinib (Fig. 2C) [28]. It is generally known that concentrations in excess of the Kd are needed to efficiently inhibit a kinase. Thus, there are even less kinases potently inhibited by dasatinib with 40 nM concentration in cardiomyocytes. This data suggests that inhibition of one or several of the kinases shown in Fig. 2C compromises cardiomyocytes viability. Further scrutiny of kinases inhibited by dasatinib at cardiotoxic concentration reveals a handful of those that have been shown to play a role in cardiovascular

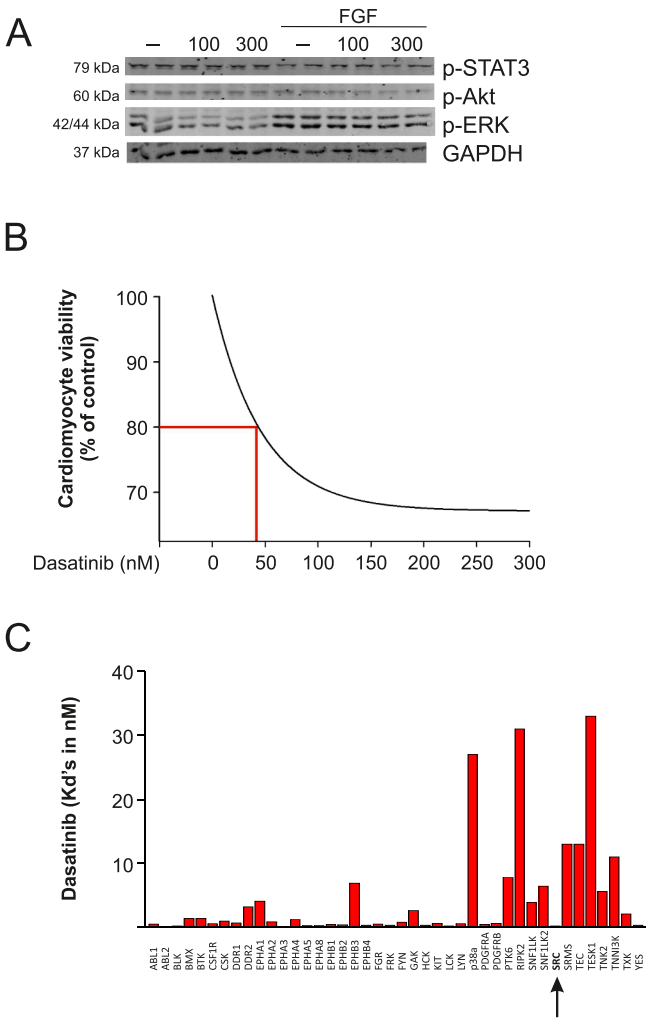

Fig. 2. *Analysis for target of dasatinib in cardiomyocytes* A. Neonatal rat cardiomyocytes were treated with 100 nM and 300 nM dasatinib for 24 h and co-treated with fibroblast growth factor (FGF) where indicated. Shown is Western blot analysis for phosphorylated STAT3, Akt and ERK. GAPHD was used as a loading control. B. Dose response curve of the cardiomyocyte toxicity of dasatinib. Cardiomyocytes were treated with 10, 30, 100, 300 and 1000 nM dasatinib for 24 h and data from ATP assay were plotted against dasatinib concentration. C. Profiling data of kinases targeted by dasatinib.

biology. One of those is Src (c-Src) that is suggested to have a prosurvival role in cardiomyocytes [29,30].

3.4. Analysis for the role of c-Src in regulating cardiomyocyte viability

To analyze for possible toxicity of Src inhibition, we targeted Src with siRNAs in cardiomyocytes. The efficiency of the target gene knockdown by RNA interference was monitored at protein level. Western blot analysis of cardiomyocytes 5 and 6 days after Src siRNA transfection showed a significant decrease in Src protein levels both at 10 nM and 30 nM concentrations (Fig. 3A). Analysis for cell viability by ATP assay showed that targeting Src significantly decreased cardiomyocyte viability (Fig. 3B). Similarly, analysis for adenylate kinase release showed an increase in cardiomyocyte death in Src siRNA transfected cardiomyocytes (Fig. 3B). Further analysis for cardiomyocyte death by measuring the release of adenylate kinase showed that Src depletion by siRNA induced cardiomyocyte death already 4 days after transfection and the toxicity was further augmented at 6 days (Fig. 3C). The data also indicated dose-dependency with higher siRNA concentration augmenting the toxicity on cardiomyocytes. In summary,

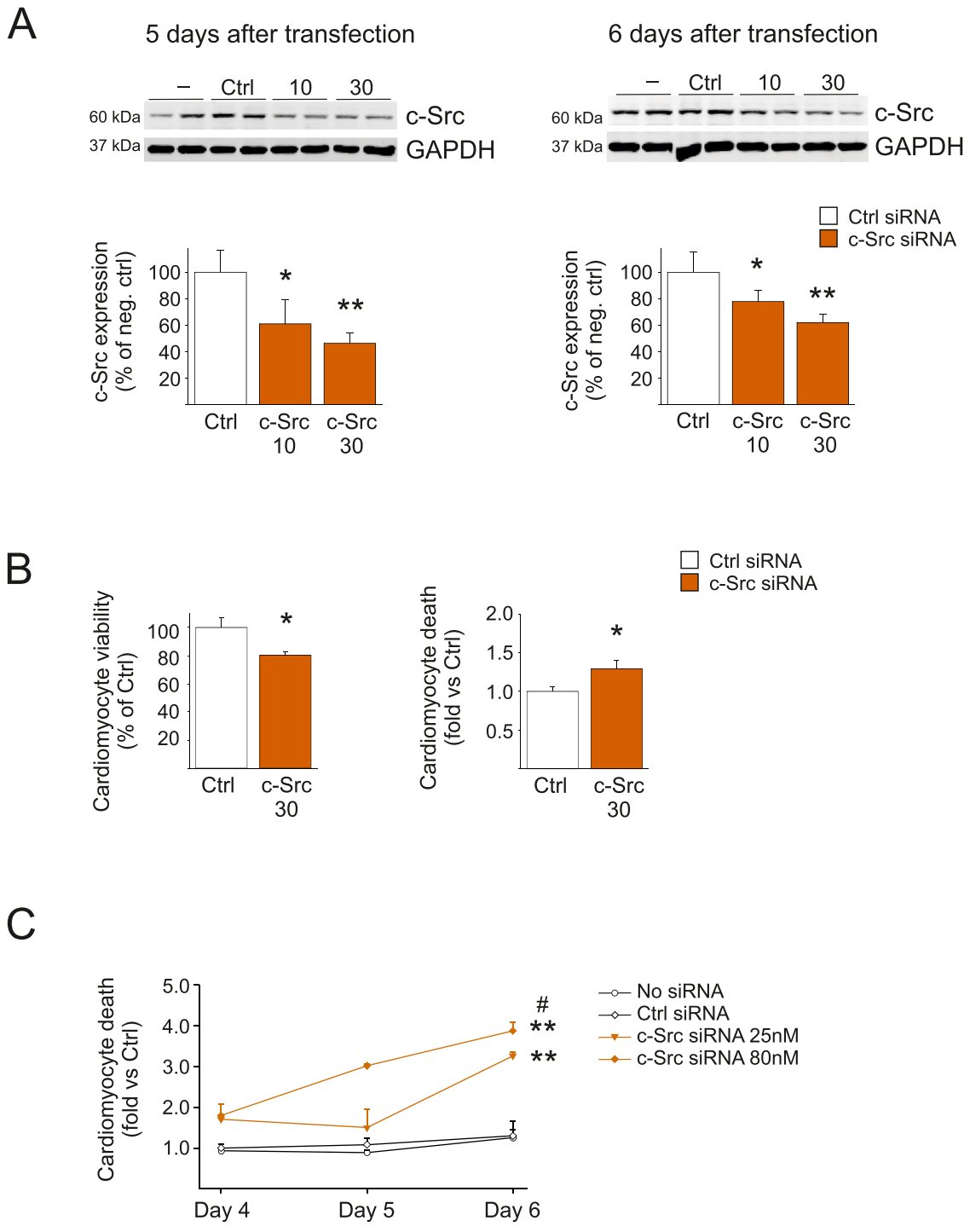

Fig. 3. *The role of c-Src in regulation of cardiomyocyte viability* A-B. Neonatal rat cardiomyocytes were transfected with control siRNA, 10 nM of c-Src siRNA or 30 nM of c-Src siRNA as indicated in the figure. A. Shown is Western blot analysis for c-Src and GAPDH. B. Shown is analysis for cardiomyocyte viability by ATP assay and analysis for cell death by measuring the release of adenylate kinase 5 days after transfection (N = 4). *p < 0.05, * *p < 0.01 vs control siRNA. Statistics were calculated by one-way analysis of variance followed by Dunnett's post hoc test. C. Cardiomyocytes were transfected with control siRNA, 25 nM of c-Src siRNA or 80 nM of c-Src siRNA as indicated. Cell death was monitored by adenylate kinase assay 4, 5 and 6 days after transfection. Data are shown as relative to adenylate kinase release from control siRNA treated cells at 4 days after transfection. * *p < 0.01 vs control siRNA; #p < 0.05 vs 25 nM c-Src siRNA. Statistics were calculated by repeated measures ANOVA.

data from these studies shows that c-Src knockdown alone compromises cardiomyocyte viability.

3.5. Dasatinib-resistant mutant of Src rescues the cardiomyocytes from the dasatinib-induced toxicity

To conclusively address the role of c-Src in dasatinib-induced cardiomyocyte toxicity, we transfected the cardiomyocytes with wild type Src and dasatinib-resistant Src (c-Src T338I; Fig. 4A). Mutation of

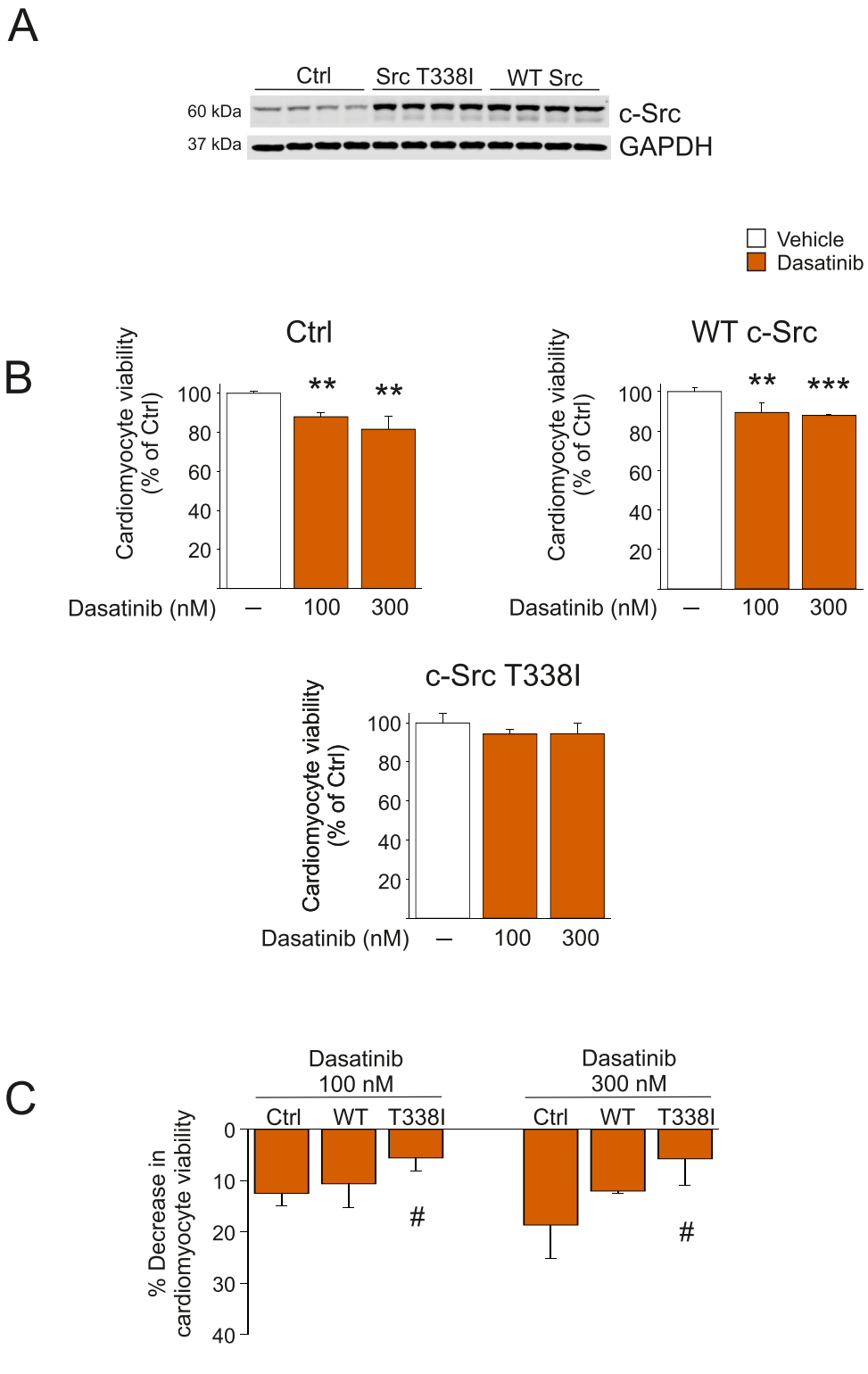

Fig. 4. *Gatekeeper mutation in c-Src rescues the cardiomyocytes from dasatinib-induced toxicity* Neonatal rat ventricular cardiomyocytes were transfected with wild type c-Src or with c-Src T338I. A. Shown is Western blot analysis for c-Src and GAPDH. B. Shown is analysis for cardiomyocyte viability by ATP assay after treatment of cells with 100 nM or 300 nM dasatinib for 24 h. N = 4. * *p < 0.01, * **p < 0.001 vs Vehicle. Statistics were calculated by one-way analysis of variance followed by Dunnett's post hoc test. C. Data shown as a comparison of toxicity of dasatinib on control cardiomyocytes and cardiomyocytes transfected with wild type c-Src or c-Src T338I. N = 4. #p < 0.05 vs control cells treated with indicated dose of dasatinib. Statistics were calculated by one-way analysis of variance followed by Tukey's post hoc test.

gatekeeper residue threonine 338 to isoleucine (T338I) converts Src resistant to dasatinib [22–24]. Analysis for cardiomyocyte viability by ATP assay showed that treatment of cardiomyocytes with 100 nM or 300 nM dasatinib reduced cardiomyocyte viability in control cells and in cells transfected with wild type Src (Fig. 4B). In contrast, dasatinib did not reduce cardiomyocyte viability in cells transfected with c-Src T338I (Fig. 4B). Comparison of dasatinib-induced toxicity between the experimental groups showed that overexpression of c-Src T338I rescued the toxicity of dasatinib on cardiomyocytes (Fig. 4C). These data thus indicate a key role for c-Src in mediating dasatinib-induced toxicity to cardiomyocytes.

4. Discussion

Src family of kinases promote cell survival, DNA synthesis and cell proliferation, whereby inhibition of Src has emerged as an attractive approach for cancer therapy [31]. Studies in the cardiovascular system have indicated a role for Src in regulating vascular function and

susceptibility to arrhythmias [32,33]. Concerning cardiomyocyte survival, studies in H9c2 rat fetal cardiomyocytes showed that overexpression of active Src was sufficient to protect the cells from doxorubicin-induced apoptosis [34]. In addition, the protective effect of metformin on doxorubicin-induced toxicity to H9c2 cardiomyocytes was abrogated by knockdown of Src [35]. Recently, Pim1 was shown to enhance cardiomyocyte viability via induction and interaction with c-Kit, which was also associated with an increase in Src protein levels [36]. At the molecular level, a variety of mechanisms are involved in Src mediated cell survival, including activation of ERK1/2, PI3K/Akt and STAT3 signaling pathways [31]. An unbiased screen utilizing kinase inhibitors recently indicated a central role for PI3k-Akt and Raf-MEK-ERK pathways promoting cardiomyocyte survival [25].

4.1. Mechanisms of cardiomyocyte toxicity of dasatinib

The extracellular signal –regulated kinase (ERK) is one of the three major mitogen-activated protein kinases (MAPKs). ERK is activated by various growth factors, cytokines, G-protein-coupled receptor ligands, and by intracellular cues. In cardiomyocytes, mechanical stretch induces rapid activation of ERK [37]. Prior studies have shown that Ras-Raf-ERK pathway is essential for cell proliferation and K-Ras is one of the most frequently activated oncogenes, with approximately 20% of all human tumors harboring an activating mutation [38]. In the myocardium, ERK has been shown to participate in the regulation of cardiomyocyte hypertrophy and cardiomyocyte survival [39]. Inhibition of ERK enhances ischemia-reperfusion –induced apoptosis, whereas increased ERK phosphorylation is associated with protection from cardiac ischemia-reperfusion injury [40,41]. Moreover, cardiomyocyte-specific overexpression of an activated MEK1 mutant, an upstream activator of ERK, induces protection from ischemia-reperfusion –induced injury [42]. Genetic reduction of ERK, or inhibition of ERK by genetic overexpression of dual-specificity phosphatase 6, a specific ERK-phosphatase, results in increased cardiac fibrosis and increased cardiomyocyte apoptosis following cardiac pressure overload [43]. These data suggest that MEK–ERK signaling protects the myocardium by either directly or indirectly antagonizing apoptosis. While the exact mechanisms for this are not fully understood [44], ERK is known to form a complex with PKCε in the mitochondria facilitating the phosphorylation and inactivation of the pro-apoptotic Bcl-2 family member Bad [45]. ERK activation in cardiac myocytes may also provide protection from apoptosis through a direct transcription linkage with GATA-4. The ERK-dependent phosphorylation of GATA-4 has been shown to offer protection from anthracycline-induced apoptosis, which was associated with Bcl-XL upregulation [46].

Here we find that treatment of cardiomyocytes with dasatinib reduces cardiomyocyte viability and reduces ERK phosphorylation. As Src kinase is one of the key targets of dasatinib and has been shown to regulate ERK activity [47,48], we assessed if Src plays a role in cardiomyocyte survival. We find that targeting Src in cardiomyocytes by RNA interference results in increased cell death, thus confirming a role for Src in regulating cardiomyocyte viability. We then investigated possible involvement of Src in dasatinib-induced cardiomyocyte death. Thr338 of Src, that is a so-called 'gatekeeper' residue, has been shown to regulate the access to a hydrophobic pocket in the ATP-binding site [22]. This is one of the most common sites of mutations in protein kinases that confer them resistant to ATP-competitive kinase inhibitors [22]. Here we find that overexpression of wild type Src is not sufficient to confer protection from dasatinib-induced decrease in cardiomyocyte viability. However, overexpression of Src containing the mutation in gatekeeper Thr338 residue of Src renders the cardiomyocytes resistant to dasatinib-induced toxicity. Western blot analysis also showed that wild type Src and dasatinib-resistant mutant of Src were expressed at similar levels further indicating that the rescue from the toxicity of dasatinib was not due to unequal expression levels Src but rather due to presence of the gatekeeper mutation in Src.

4.2. Toxicity of dasatinib on endothelial cells and fibroblasts

As dasatinib may also induce injury to the myocardium by reducing the viability of other resident cardiac cells, we also analyzed the toxicity of dasatinib on endothelial cells and fibroblasts. We find that dasatinib has negligible effect on viability of fibroblasts. This is of importance since neonatal rat cardiomyocyte cultures (if not prepared with Percoll gradient) include fibroblasts. As dasatinib did not induce adenylate kinase release from fibroblasts, it is conceivable that the toxicity of dasatinib in the cardiomyocyte cultures indeed stems from the toxicity to the cardiomyocytes. On the other hand, treatment of endothelial cells with dasatinib results in dose-dependent increase in cell death. This adverse effect of dasatinib on endothelial cells is consistent with the previous data [49] and may potentially play a role in the increased risk of pulmonary hypertension in patients treated with dasatinib. Disturbances in endothelial cell homeostasis in the myocardium also adversely affects the function and integrity of adjacent cardiomyocytes and may exacerbate the cardiotoxic effects of dasatinib.

Cardiotoxicity of cancer therapeutics is clinical problem and a major aspect analyzed for during drug development. Understanding the mechanisms of toxicity is necessary for predicting and preventing cardiotoxicity of kinase inhibitors in patients and could enable identifying the kinases whose targeting is to be avoided when possible in the design of novel KIs. Here we find that dasatinib induces cardiomyocyte toxicity via inhibition of c-Src, which may have an impact on future drug design and monitoring of cardiovascular status of patients treated with agents targeting c-Src.

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CRediT authorship contribution statement

Manar Elmadani: Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – review & editing. **Sami Raatikainen:** Formal analysis, Funding acquisition, Investigation, Methodology, Visualization, Writing – review & editing. **Orvokki Mattila:** Formal analysis, Investigation, Methodology. **Tarja Alakoski:** Funding acquisition, Investigation, Methodology. **Jarkko Piuhola:** Data curation, Formal analysis, Investigation, Methodology. **Pirjo Åström:** Formal analysis, Investigation, Methodology. **Olli Tenhunen:** Formal analysis, Investigation, Writing – review & editing. **Johanna Magga:** Conceptualization, Funding acquisition, Investigation, Project administration, Supervision, Visualization, Writing – review & editing. **Risto Kerkelä:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project Administration, Supervision, Visualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.toxrep.2023.04.013.

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